

reversal) would allow the sensitive separation of the specific products of target-dependent cleavage from probe fragments generated by thermal degradation was examined.

To test the sensitivity limit of this detection method, the target M13mp18 DNA was serially diluted ten fold over than range of 1 fmole to 1 amole. The invader and probe oligonucleotides were those described above (*i.e.*, SEQ ID NOS:61 and 62). The invasive cleavage reactions were run as described above with the following modifications: the reactions were performed at 55°C, 250 mM or 100 mM KGlu was used in place of the 100 mM KCl and only 1 pmole of the invader oligonucleotide was added. The reactions were initiated as described above and allowed to progress for 12.5 hours. A negative control reaction which lacked added M13mp18 target DNA was also run. The reactions were terminated by the addition of 10 µl of 95% formamide containing 20 mM EDTA and 0.02% methyl violet, and 5 µl of these mixtures were electrophoresed and visualized as described above. The resulting imager scan is shown in Figure 61.

In Figure 61, lane 1 contains the negative control; lanes 2-5 contain reactions performed using 100 mM KGlu; lanes 6-9 contain reactions performed using 250 mM KGlu. The reactions resolved in lanes 2 and 6 contained 1 fmole of target DNA; those in lanes 3 and 7 contained 100 amole of target; those in lanes 4 and 8 contained 10 amole of target and those in lanes 5 and 9 contained 1 amole of target. The results shown in Figure 61 demonstrate that the detection limit using charge reversal to detect the production of specific cleavage products in an invasive cleavage reaction is at or below 1 attomole or approximately 6.02×10^5 target molecules. No detectable signal was observed in the control lane, which indicates that non-specific hydrolysis or other breakdown products do not migrate in the same direction as enzyme-specific cleavage products. The excitation and emission maxima for Cy3 are 554 and 568, respectively, while the FMBIO Imager Analyzer excites at 532 and detects at 585. Therefore, the limit of detection of specific cleavage products can be improved by the use of more closely matched excitation source and detection filters.

EXAMPLE 24

Devices And Methods For The Separation And Detection Of Charged Reaction Products

This example is directed at methods and devices for isolating and concentrating specific reaction products produced by enzymatic reactions conducted in solution whereby the reactions generate charged products from either a charge neutral substrate or a substrate bearing the opposite charge borne by the specific reaction product. The methods and devices of this example allow isolation of, for example, the products generated by the invader-directed cleavage assay of the present invention.

The methods and devices of this example are based on the principle that when an electric field is applied to a solution of charged molecules, the migration of the molecules toward the electrode of the opposite charge occurs very rapidly. If a matrix or other inhibitory material is introduced between the charged molecules and the electrode of opposite charge such that this rapid migration is dramatically slowed, the first molecules to reach the matrix will be nearly stopped, thus allowing the lagging molecules to catch up. In this way a dispersed population of charged molecules in solution can be effectively concentrated into a smaller volume. By tagging the molecules with a detectable moiety (*e.g.*, a fluorescent dye), detection is facilitated by both the concentration and the localization of the analytes. This example illustrates two embodiments of devices contemplated by the present invention; of course, variations of these devices will be apparent to those skilled in the art and are within the spirit and scope of the present invention.

Figure 62 depicts one embodiment of a device for concentrating the positively-charged products generated using the methods of the present invention. As shown in Figure 62, the device comprises a reaction tube (10) which contains the reaction solution (11). One end of each of two thin capillaries (or other tubes with a hollow core) (13A and 13B) are submerged in the reaction solution (11). The capillaries (13A and 13B) may be suspended in the reaction solution (11) such that they are not in contact with the reaction tube itself; one appropriate method of suspending the

capillaries is to hold them in place with clamps (not shown). Alternatively, the capillaries may be suspended in the reaction solution (11) such that they are in contact with the reaction tube itself. Suitable capillaries include glass capillary tubes commonly available from scientific supply companies (*e.g.*, Fisher Scientific or VWR Scientific) or from medical supply houses that carry materials for blood drawing and analysis. Though the present invention is not limited to capillaries of any particular inner diameter, tubes with inner diameters of up to about 1/8 inch (approximately 3 mm) are particularly preferred for use with the present invention; for example Kimble No. 73811-99 tubes (VWR Scientific) have an inner diameter of 1.1 mm and are a suitable type of capillary tube. Although the capillaries of the device are commonly composed of glass, any nonconductive tubular material, either rigid or flexible, that can contain either a conductive material or a trapping material is suitable for use in the present invention. One example of a suitable flexible tube is Tygon® clear plastic tubing (Part No. R3603; inner diameter = 1/16 inch; outer diameter = 1/8 inch).

As illustrated in Figure 62, capillary 13A is connected to the positive electrode of a power supply (20) (*e.g.*, a controllable power supply available through the laboratory suppliers listed above or through electronics supply houses like Radio Shack) and capillary 13B is connected to the negative electrode of the power supply (20). Capillary 13B is filled with a trapping material (14) capable of trapping the positively-charged reaction products by allowing minimal migration of products that have entered the trapping material (14). Suitable trapping materials include, but are not limited to, high percentage (*e.g.*, about 20%) acrylamide polymerized in a high salt buffer (0.5 M or higher sodium acetate or similar salt); such a high percentage polyacrylamide matrix dramatically slows the migration of the positively-charged reaction products. Alternatively, the trapping material may comprise a solid, negatively-charged matrix, such as negatively-charged latex beads, that can bind the incoming positively-charged products. It should be noted that any amount of trapping material (14) capable of inhibiting any concentrating the positively-charged reaction products may be used. Thus, while the capillary 13B in Figure 62 only contains trapping material in the lower, submerged portion of the tube, the trapping material